

## THE ROLE OF PROBIOTICS IN AQUACULTURE IN NIGERIA– A REVIEW

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### ABSTRACT

Aquaculture is beset by many problems especially diseases caused by bacteria as the major deteriorating factors. The use of vaccines and antimicrobial agents have been centered on disease control, but are associated with problems. The development of antibiotic resistance among the microorganisms have become a global concern as a result of indiscriminate use of antibiotics. Several alternative suggestions for disease prevention have been on probiotics for its efficacy, low cost, less side effects and accessible to farmers. Probiotics is gaining a high priority in the developed countries with the aim of replacing conventional drugs. The principal bacterial groups tested as probiotic bacteria in culture of shrimps, crabs, oysters, fish and humans are *Vibrio*, *Pseudomonas*, *Bacillus*, *Bifidobacteria* and several *Lactobacilli*. Experiments have mainly been conducted with fish larvae, adult fish, crustaceans and animals where significant reduction in mortalities has been obtained. The purpose of this review is to create awareness of the role of probiotics in disease control in aquaculture as alternative to antibiotics.

**KEYWORDS:** Role, Probiotics, Disease control, Aquaculture, Nigeria

### INTRODUCTION

Aquaculture is concerned with the propagation and rearing of aquatic organisms under complete human control involving manipulation of at least one stage of an aquatic organism's life before harvest in order to increase its production. This practice has become an important economic activity in many countries. In large-scale production facilities where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental condition often occur and result in serious economic losses. Prevention and control of diseases through the use of antibiotics and vaccines have been in practice in many countries of the world. However the utility of antimicrobial agents as a preventive measure has been questioned, given extensive documentation of the evaluation of antimicrobial resistance among pathogenic bacteria. The problems from the use of antibiotics have attracted a global concern in terms of development of resistance among the pathogenic bacteria. Resistance mechanisms of bacteria can arise in one of two ways: chromosomal mutation or acquisition R – plasmid.

There is therefore an urgent need in aquaculture development for microbial control strategies since disease outbreaks are recognized as important constraints to aquaculture and despite the fact that the development of antibiotic resistance has become a matter of growing concern. One alternative to antibiotics in disease control in aquaculture could be the use of probiotic bacteria. The use of probiotics or beneficial bacteria which control pathogen through a variety of mechanisms is increasingly viewed as an alternative to antibiotic treatment. The use of probiotics in human (Anukam, *et al.*, 2004, 2006 and Anukam, 2007) in animal nutrition (Fuller, 1992) are well documented and recently they have begun to be applied in aquaculture (Gatesoupe, 1999; Gomez-Gill *et al.*; 2000, Veschuere *et al.*, 2000, Irianto and Austin, 2002).

### POTENTIAL PROBIOTIC BACTERIA IN USE

Probiotics are harmless bacteria that help the wellbeing of the host animal and contribute directly or indirectly to protect the host animal against harmful bacterial pathogens. A review indicates a considerable potential of certain harmless bacterial strains to prevent or control fish diseases caused by pathogenic bacteria as well as boosting growth performance in farmed fish (Ashraf, 2000). It is assumed that uncontrolled development of the microbial communities in hatcheries is one of the major reasons for the unpredictable and often variable results; the introduction of microbial control practices by means of Probiotics may have a beneficial effect on the cultures in the hatcheries. Griffith (1995) reported that following the introduction of Probiotics in Ecuador in

1992, hatchery down-times between batches was reduced from 7days per month to 21 days annually, production volume increased by 35% and over all antimicrobial use decreased by 95%.

In agriculture the value of probiotics notably Gram-positive bacteria such as *Lactobacillus* has come to be appreciated as an alternative to antibiotics in disease control. A wide variety of micro-organisms have been found capable of producing substances which are inhibitory to other organisms. Other organism e.g. *Pseudomonas* are also known to be a natural competitor to other organisms like *Saprolegnia* (Bly, *et. al.* 1997). These organisms that have the ability to compete, produce substance that stimulate the growth of other organisms or inhibit the growth of another are probiont and in future may be utilized for biological control in aquaculture as in mammalian probiotics. It is there for important to consider those bacteria that have antagonistic effect and those bacteria that stimulate the growth of others as important as potentials for probiotics in aquaculture. Einar (2002) reported that the most promising organisms for probiotic are *Vibrio* and *Pseudomonas* for fish and *Bacillus* for shrimp systems. Bergh (1995) added that the isolates that inhibit the growth of other bacteria belong to *Pseudomonas* /*Alteromonas* group, and the ability to produce compound that inhibit the growth of other bacteria may be wide spread among marine and fresh water bacteria. Austin *et al.* (1995) reported that a strain of *V. alginolyticus* was effective in reducing disease caused by *Aeromonas salmonicida* and 2 pathogenic *Vibrio* species. The table below summarizes the common probiont employed in culture of different aquatic organisms according to Gomez-Gill *et al.* (2000)

Table 1: Probiotics employed in culture of aquatic organisms

Species of bacteria	Target organism	Reference
<i>Vibrio alginolyticus</i>	Shrimp ( <i>Panaeus vannamei</i> )	(Garriques and Arevalo, 1995)
<i>Thalassobacter utilis</i> (PM-4)	Shrimp ( <i>P. monodon</i> )	Maeda and Liao, 1991)
<i>V. harveyi</i> , <i>Pseudomonas</i> sp., <i>Nitrobacter</i> sp <i>Nitrosomonas</i> sp., and <i>Bacillus</i> sp.	Shrimp ( <i>P. monodon</i> and <i>P. penicillatus</i> )	(Anonymus, 1991)
<i>T. utilis</i> (PM-4)	Crab ( <i>Portunus trituberculatus</i> )	(Nogami and Maeda, 1992, Nagomi, <i>et.al.</i> 1997)
<i>V. Pelagius</i>	turbot ( <i>Scophthalmus maximus</i> )	(Ringo and Vadstein, 1998)
<i>Bacillus toyoi</i> and <i>B. sp. spore</i>	Turbot via rotifers ( <i>Branchionus plicatilis</i> )	(Gatesoupe, 1989, )
<i>Lactic bacteria</i>	Turbot via rotifers	(Gatesoupe, 1990)
<i>Lactobacillus plantarum</i> and <i>L. helveticus</i>	Turbot via rotifers	(Gatesoupe, 1991)
<i>Lacobcillus bulgaricus</i> and <i>Streptococcus lactis</i>	Turbot via Artemia	(Douillet and Langdon, 1994)
<i>Aleromonas</i> sp.	Oyest ( <i>Crossotrea gigers</i> )	(Douillet and Langdon, 1994)
<i>Aeromonas media</i>	Oyster	(Gibson, <i>et.al.</i> 1998)
<i>Rosebacter</i> sp (NS107)	Scallop ( <i>Pecten maximus</i> )	(Ruiz-Ponte, <i>et.al.</i> 1999)
<i>Vibrio</i> sp	Chalean scallop ( <i>Argopecten purpuratus</i> )	(Riquelme <i>et.al.</i> 1997)

Source: Gomez- Gill *et al.* 2000

The highly researched and most used probiotics according to Berger (2002), are *Lacobacillus acidophilus*, *L. bulgericus*, *Bifidobacteria longum* and *B.infantis* Some of the commercial probiotics currently available in use include Aqualact, probela, Lacto-sacc Epicin, Biogreen, Environ, Wunopuo-15 and Epizyme (Abidi, 2003). However evidences indicate that probiotic effects are strain-specific, therefore a beneficial effect attributed to one strain cannot be assumed by another strain, even when they belong to the same species (Senok, *et.al.* 2005). Table 2 shows the probiotic bacteria used in aquaculture fishes.

Table 2: Probiotics considered as biological control agents in aquaculture of fishes

Probiotic strain	Source	Used on	Method of application	Reference
<i>Streptococcus lactis</i> and <i>Lactobacillus bulgaricus</i>	?	Turbot larvae ( <i>Scophthalmus maximus</i> )	Enrichment of live food	Garcia de la Blanda <i>et al.</i> (1992)
<i>Lactobacillus</i> sp. and <i>Carnobacterium</i> sp.	Rotifers ( <i>Brachionus</i> <i>plicatilis</i> )	Turbot larvae	Enrichment of rotifers	Gatesoupe, <i>et al.</i> (1995)
<i>Vibrio alinolyticus</i>	commercial shrimp Hatchery	Atlantic salmon ( <i>Salmosalar</i> )	Bathing in bacterial suspension	Austin, <i>et al.</i> (1992)
<i>Carnobacterium divergens</i>	Intestine of Atlantic salmon	Atlantic cod fry	Addition diet	Gibson <i>et al.</i> (1998)
<i>Bacillus megaterium</i> <i>B. subtilis</i> , <i>B. polymyxa</i> <i>B. licheniformis</i>	commercial product (Biostart)	Channel catfish	Additid to pond water	Queeirz and Boyd (1998)
<i>Vibrio pelagius</i>	Turbot larvae	Turbot	Addition to culture water	Ringo and Vadstein (1998)
<i>Pseudomonas fluorescens</i>	Iced freshwater fish ( <i>Lates niloticus</i> )	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Addition to culture water	Gram <i>et al.</i> (1999)
<i>Lactobacillus rhamnosus</i>	culture collection	Rainbow trout	Addition to diet	Nikoskelainen <i>et al.</i> (2001)
<i>Aeromonas hydrophila</i>	Digestive tract of rainbow trout	Rainbow trout	Addition to diet	Irianto and Austin, (2002)
<i>Bacillus circulans</i>	Intestine of <i>Labeo rotifer</i>	<i>L. rotifer</i>	Addition to diet	Gomez-Gil, <i>et al.</i> (2000)

Source: Balcazar *et al.* (2006)

## APPLICATION OF PROBIOTICS IN AQUACULTURE

These organisms can be administered to the aquaculture organisms through feeding, injection or immersion of the probiotic bacteria (Irianto and Austin, 2002).

### Application in Feed

Probiotics are applied with the feed and a binder (egg or cod liver oil) and most commercial preparation contain either *Lactobacillus sp* or *Sacharomyces cerevisiae* (Abidi, 2003). Regular use of probiotic in feed of fish in U.K. and other European countries has been reported to have several health benefits (Cerrato 2000).

The perception that fermented milk yoghurt is beneficial is already wide spreading within so many regions because, traditionally these products have been used by local healers for the treatment of diverse condition, such as skin, allergies, stomach upset especially diarrhea and vaginal discharges.

According to FAO and WHO guidelines, probiotic organisms used in food must be capable of surviving passages through the gut i.e. they must have the ability to resist gastric juices and exposure to bile (Senok *et al* 2005). Furthermore they must be able to proliferate and colonize the digestive tract and they must be safe, effective and maintain their effectiveness and potency for the duration of the shelf life of the product (Senok *et al*, 2005).

Dairy products including yoghurt, fermented milk product and cheese remain at forefront of probiotic food development in humans. Yoghurt with added live probiotic strains is now available commercially and a number of such products have emerged as leaders in the European market are now also available internationally. In aquaculture probiotic can also be encapsulated in feed (Einar 2002) or through live food like rotifers and artemia. Another efficient application of probiotics to aquatic animal according to Einar (2002) is via bio-encapsulation or infusions in diets. Preparation of probiotic diet has been demonstrated by Yassir *et al*, (2002).

### Direct to Culture Water/ Pond

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. lecheniformis*, *Nitrobacter sp*, *Aerobacter* and *Sacharomyces cerevisiae*. Application of probiotic through water of tanks and ponds may also have an effect on fish health by improving several qualities of water, since they modify the bacteria composition of the water and sediments (Ashraf, 2000; Venkateswara, 2007). When probiotics are applied in culture water they multiply and over grow the pathogenic organism present in the water. Beside this Venkateswara (2007) reported that probiotic bacteria are generally called bacteria which can improve the water quality of aquaculture and inhibit the pathogens in water thereby increasing production.

Today many researchers attempt to use some kind of probiotic in aquaculture water to regulate the microflora of aquaculture water, control pathogenic microorganism to enhance decomposition of undesirable organic substances in the water and improve ecological environment of aquaculture (Xiang-Hong *et al*;2000 Venkateswara ,2000). Einar (2002) identified some ways of using probiotics either as larval food or enriched brachionus or artemia with probiotics.

### Application through Injection

Application of probiotics by injection is a possibility. Austin, *et al*. (1995) suggested the possibility of freeze-drying the probiont like vaccine and applied either through bathing, or injection. Except for experimental purposes application of probiotic by injection has not been widely reported. Even though one of the benefits of probiotics in fish and animal is to boost the immunity, meaning that there is the stimulation of antibodies of the host. Probiotics can therefore confer the best immunity through injection as Lamar (1985) reported that injection the best method of vaccination. However (Gram *et al* (1999) state that vaccination by injection which some times the only effective route of administration is impracticable when applied to small fish or larger numbers. Yassir *et al*. (2002) has demonstrated the experimental administration of probiotic *Micrococcus luteus* to *Oreochrmis niloticus* by injection through intra peritoneal route which had only 25% mortality as against 90% with *Pseudomonas* using the same route. According to Yassir *et al* (2002) the use of probiotics stimulate *Rainbow trout* immunity by stimulating phagocytes activity, complement mediated bacterial killing and immunoglobulin production (Nikoselainen *et al*, 2003). When probiotics is evaluated in freeze dried form, it can be applied through injection

## BENEFITS OF PROBIOTICS

Although some of the effects of probiotics have been documented clearly, research is still on going in the area with so many questions on the reality of some of the benefit remaining unanswered. However it is crucial to remember that different probiotic strains are associated with different health benefits (Senok *et al.*2005).

### Improvement in Water Qualities

According to Venkateswara (2007), probiotics have been reported to regulate micro flora, control pathogenic ones, enhances the decomposition of the undesirable organic substance, improve ecological environment by minimizing the toxic gasses like NH<sub>3</sub>, N<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>, Methane etc, increases population of food organism in the water, increases nutritional level of the aquatic host and improve their immunity in the culture water. In several studies, improved water quality has been recorded during the addition of the probiotics especially with *Bacillus sp.* (Verschuere *et al*, 2000). The rationale is that Gram-positive *Bacillus sp.* are generally more effective in converting organic matter back to CO<sub>2</sub> than G-negative bacteria which could convert a greater percentage of organic carbon to bacterial biomass or slime.

### As Growth Promoters

One of the activities of probiotic bacteria is expected to have a direct growth promoting effect of fish either by direct involvement in nutrient uptake or by providing nutrient or vitamin. However, it has been demonstrated experimentally that probiotics indeed may enhance the growth of fish. The ability of organisms to out-grow the pathogens in favor of host or to improve the growth of the host and yet no side effect on the host made it a probiotic bacteria. Yassir *et al.* (2002) in attempt to use probiotic bacteria as growth promoter on tilapia (*Oreochromis niloticus*) identified that the highest growth performance was recorded with *Micrococcus luteus* a probiotic and the best feed conversion ratio was observed with the same organism. So *M. luteus* may be considered as a growth promoters in fish aquaculture. Lactic acid bacteria also had an effect as growth promoters on the growth rate in juvenile carp though not in Sea bass (Noh *et al*, 1994). Also *Enterococcus faecium* had been used to improve growth when applied in feed to fish (Bogut *et al.* 2000). Irianto and Austin (2002) reported that probiotics may stimulate appetite and improve nutrition by producing vitamins, detoxification of compounds in the diet and by the breakdown of indigestible components. *Streptococcus faecium* improved the growth and feed efficiency of *Israeli carp* (Noh *et al.* 1994). Probiotics therefore can be regarded as growth promoters in aquaculture organisms in addition to various benefits of probiotics.

### For Good Health

Many probiotic products are used routinely by healthy individuals indeed the marketing of many probiotics food probiotics is targeted at healthy individuals. The claim that regular ingestion will contribute to a healthy life style, promote general wellbeing and protect against or reduce the risk of developing chronic gastrointestinal, respiratory or cardiac problems in the long term has induced many people to undertake regular consumption of these products. (Senok, *et.al.*, 2005). Selected probiotics have shown to have significant health benefits for humans and thus several well-characterized strains are available for human use to reduce the risk of gastrointestinal infection or to treat such infection (Nikoskelainen, 2001). In countries of continental Europe, probiotics are regarded as medicine and they are prescribed alongside antibiotics, while in other countries probiotics are marketed as supplement and are sold over the counter (Berger, 2002) for good health.

### For Disease Prevention

Probiotics or their products for health benefits to the host have been found useful in aquaculture, terrestrial animals and in human disease control. These include microbial adjunct that prevent pathogens from proliferating in the intestinal tract, on the superficial surfaces and in culture environment of the culture species (Verschuere, *et.al.* 2000). The effect of these beneficial organisms is achieved through optimizing the immune system of culture organism, increasing their resistance to disease, or producing inhibitory-substance that prevent the pathogenic organisms from establishing disease in the host. Culture System, Inc, (2002) added that good bacteria in the gut aid in many important function such as nutrient digestion, immune function and prevention of pathogens.

One of the well established benefits of probiotics is the decrease in occurrence and duration of diarrhea especially those caused by antibiotics like ampicillin using mixture of *L. bulgericus*, *Lacidophilus*, *Bifidobacterium* and *Streptococcus* bacteria. This is also true for the use of *L. rhamnosus* GG, *L. reuteri* SD2222 and *B. lactis* BB-12 in the prevention and treatment rotavirus diarrhea in children, cited by Senok *et.al*

(2005). However probiotics is becoming a popular treatment for the occurrence of urogenital infection in the women. Several studies suggest that the administration of *Lactobacillus* either orally or intravaginally is an effective treatment and preventive measures against urogenital infection (Geldzwis, 2007 and Senok, *et al.* 2005). Some authors conducted studies in animals showed that probiotics may be a protective factor against cancer of colon, prostate, bladder and other cancer, because probiotic bacteria protect carcinogenic activities and *L. acidophilus* or *L. casei* are reported to play this role. Therefore the use of probiotics have a potential benefits for conditions such as gastrointestinal infection, genitourinary infection, allergies and certain bowel disorders all which afflict a considerable proportion of the global population (Senok *et al.* 2005).

#### SELECTION CRITERIA FOR PROBIOTICS

The initial major purpose of using probiotics is to maintain or re-establish a favorable relationship between friendly and pathogenic microorganism that constitute the flora of intestinal or skin mucus of fish. A successful probiotic is expected to have a few specific properties in order to certify, a beneficial effect.

FAO/WHO (2001) guidelines recommend that in the cause of selecting probiotics, the probiont should be evaluated for a number of parameters such as, antibioticsusceptibility patterns, toxic production, metabolic and hemolytic activities, infectivity in immuno-compromised host and side-effects

#### Characteristics of Good Probiotics

Filler (1989) listed the following as features of good probiotic bacteria.

- Its should be a strain, which is capable of exerting a beneficial effect on the host animal e.g. increased growth or resistance to disease
- It should be non-pathogenic and non-toxic
- It should be present as viable cells preferable in large numbers
- It should be capable of surviving and metabolizing in the gut environment e.g. resistance to low pH and organic acid.
- It should be stable and capable of remaining viable for periods under storage and field conditions.

A probiotic agent with all these features has considerable advantage over antibacterial supplements such as antibiotics currently in use. They do not induce resistance to antibiotics which will compromise therapy. They are not toxic and therefore will not produce undesirable side effect when being feed and in the case of food animal will not produce toxic residues in the carcass. They may stimulate immunity whereas the immune status remains unaffected by antibiotics. An essential determinant in the choice of a probiotic microorganism is its ability to reach, survive and persist in the environments (Charles *et al.* 1998).

#### CONCLUSION

Probiotics as an alternative to antibiotics in aquaculture becomes imperative. The most highly researched probiotic bacteria today are *Lactobacillus acidophilus*, *L. bulgarium*, *Bifidobacteria longum*, and *B. infantis*

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